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Sampling of *Lumbricus terrestris* (Oligochaeta, Lumbricidae)

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With 2 figures

Synopsis: Original scientific paper

The extraction of juveniles of *L. terrestris* was equally efficient by the application of formaldehyde or chloroacetophenone (CN) solutions and by a chloroacetophenone application followed by a handsorting to a soil depth of 110 cm. For adults the combination CN/handsorting was more efficient than the use of chemical expellants only. The efficiency of chemical expellents for the extraction of adults may have been improved through a repeated application of the chemicals on three subsequent evenings.

The frequency distribution of juveniles could be described by a negative binomial, that of the adults by a Poisson distribution. These statistical distributions were used to calculate the sample size for a given reliability. It is concluded that if adult and juvenile *L. terrestris* are abundant, their population densities are reliably estimated in a homogeneous environment by taking 10 sample units of 50 cm × 50 cm per sample. However, in non-homogeneous environments or in habitats with low earthworm population density, the appropriate sample size is expected to be much higher. Therefore, further investigations should not only concentrate on the elevation of the control of the extraction efficiency, but also should aim to reduce the time needed for the sampling.

Key words: *Lumbricus terrestris*, sampling statistics, earthworm sampling, extraction techniques.

1. Introduction

Several techniques for extracting earthworms from soil have been described (e.g. BOUCHÉ, 1969; SATCHELL, 1969; LEE, 1985; THIELEMAN, 1986); each has advantages and disadvantages in terms of use and efficiency of extraction. Chemical solutions used as expellants produce rapid results compared to handsorting. The formaldehyde method, first published by RAW (1959), has been used by several authors (e.g. BOUCHÉ, 1969; SATCHELL, 1969; NORDSTRÖM & RUNDGREN, 1972; SPRINGETT, 1981; TERHIVUO, 1982; BAKER, 1985). It is well known that the efficiency of this method is subject to circadian (KRETZSCHMAR, 1982; DANIEL, 1986) and seasonal (BOUCHÉ & GARDNER, 1984) effects. SATCHELL (1969) proposed a model for correcting seasonal changes in the sampling efficiency of *Lumbricus terrestris* L., but the model was validated with only one sample unit.

Various arbitrary criteria have been used for the appropriate sample size (i.e. number of sample units) for estimating earthworm population densities, e.g. continuing the sampling until approximately 1000 ind. of *L. terrestris* had been collected (LAKHANI & SATCHELL, 1970) or collecting earthworms in 16 sample units of 25 cm × 25 cm (ZICSI, 1962). DICKEY & KLADIVKO (1989) used handsorting to compare different shapes and sizes of sample units in crop lands. However, their data set was obtained from a systematic sampling along transects and statistical inferences about requisite sample sizes are based on the assumptions that the counts of earthworms are Poisson distributed.

Inefficiencies in the extraction with expellants may be caused by deleterious effects of chemicals (e.g. on motility), by inadequate levels of exposure to the irritant substance, and

by factors hampering the ability of earthworms to respond to the expellant (e.g. low temperature, dormancy). In this study the extraction efficiency of formaldehyde (which has deleterious and irritant effects) was compared with chloroacetophenone (CN) (which was assumed to mainly have irritant properties at the concentrations used), and the application of CN followed by handsorting. Measures which might improve the exposure of *L. terrestris* to irritant substances were tested as well. Samples were taken randomly in a defined area and the optimum sample size for the estimation of population densities of *L. terrestris* was determined for a reliability defined in statistical terms.

2. Material and methods

2.1. Experiments

2.1.1. Location

Experiments were carried out in a level meadow (part of a plot named "Chaiblen") near the Swiss Federal Research Station for Farm Management and Agricultural Engineering (FAT) in Tänikon (canton of Thurgau, Switzerland). The soil is reported to be a loamy, partially decarbonated, slightly pseudogleyic brown soil (ANONYMOUS, 1971). An area of 1600 m² was divided into quadrants I, II, III, IV. Parts of the area, especially in quadrants II and IV, were filled several decades ago with stony soil.

2.1.2. Treatments

Two experiments were carried out: **Experiment 1** (tab. 1) was conducted to investigate the efficiency of the application of the chemical expellants formaldehyde and chloroacetophenone (CN) as compared to a CN application followed by handsorting. In treatments A and B, 20 to 60 liters per 0.25 m² were needed to pond a surface delimited by metal box quadrats of 50 cm × 50 cm for at least 40 min. Metal box quadrats had a height of 10 cm and were pressed 2–3 cm into the soil. Earthworms were collected during ponding and within 60 min thereafter within metal rings of 40 cm diameter (treatment A) or within the metal box quadrats (treatment B). In treatment A the extraction by means of CN was followed by handsorting. For this purpose the soil beneath the metal rings was sampled with a corer (40 cm diameter) to a depth of 110 cm. Earthworms collected in treatment A (CN/handsorting) were pooled. In the treatments C and D the formaldehyde (0.1 and 0.2%) was applied as described by

Table 1. Treatments used for expelling *Lumbricus terrestris* in experiment 1 and 2 (FA: formaldehyde, CN: chloroacetophenone).

treat- ment	expel- lant	concen- tration (%)	volume	sample unit (m ²)	time of day	date 1986
experiment 1						
A*	CN	0.008	ponded	1/8	16-19	17.06
B	CN	0.005	ponded	1/4	16-19	19.06
C	FA	0.1	2 × 5 l	1/4	08-10	10.06
		0.2	2 × 5 l			
D	FA	0.1	2 × 5 l	1/4	17-19	10.06
		0.2	2 × 5 l			
experiment 2						
E	CN	0.005	ponded	1/4	16-19	07.11
F	CN	0.0002	ponded	1/4	17-22	04.11
		0.002	ponded		17-22	05.11
		0.005	ponded		17-22	06.11

* treatment A included a handsorting as specified in the text

BOUCHÉ (1969). **Experiment 2** (tab. 1) was carried out to test whether a set of measures which are assumed to improve the exposure of the earthworms to the irritant substance affects the extraction efficiency. Treatment E was the same as treatment B in experiment 1 (tab. 1). In treatment F quadrats were covered between the three consecutive extractions. The pondings were accomplished within an inner (0.5 m × 0.5 m) and an outer (1 m × 1 m) metal quadrat box, but earthworms were only collected in the inner quadrat. The rationale was to improve the wetting of the soil by a repeated ponding of an inner and outer area (analogous to a double ring infiltrometer) and to apply the irritant at a time of day when *L. terrestris* is active and comes to the surface.

Five sample units, whose position within the quadrants was determined by means of random numbers, were taken for each quadrant and each treatment.

2.1.3. Preservation and identification of the earthworms

Earthworms were killed and preserved using 4% formaldehyde. The preservative solution was renewed after one month.

Both *L. terrestris* and *L. castaneus* were present in the meadow under investigation. *L. terrestris* with visible male pores were classified as adults. Immatures of *Lumbricus* sp. which were smaller than adult *L. castaneus* were assigned to *L. castaneus* if no pigmentation gradient was visible along the longitudinal axis.

2.2. Analysis of the experiments

2.2.1. Effects of quadrants and treatments

The level of significance was fixed for all statistical analyses at a probability (p) ≤ 0.05 . Earthworm numbers in treatment A were multiplied by 2, because the sample unit was only half of that in the other treatments. This was justified on the basis that preliminary studies with sample units of 1/8 and 1/16 m² showed no influence of the size of the sample unit on the proportion of the mean to the variance of *L. terrestris*. Two-way analyses of variance (LINDER & BERCHTOLD, 1982; ZAR, 1984), with the data of treatments A–D with factors "treatment" and "quadrant", were conducted separately for adults and juveniles. Data used for the two-way analyses of variance were transformed to improve homoscedasticity according to equations [1] and [2] (ELLIOTT, 1977) for adults and juveniles, respectively:

$$x^* = \sqrt{x} \quad [1]$$

where

x^* : transformed value of x ,

x : number of adults per 0.25 m²,

and

$$x^* = \sinh^{-1} \sqrt{\frac{x + 0.375}{k - 0.750}} \quad [2]$$

where

x^* : transformed value of x ,

x : number of juveniles per 0.25 m²,

k : parameter of the negative binomial distribution (see equation [3]).

Two-way analyses of variance were made to determine whether the effect of the quadrants or of the treatments was significant. If the effect of a factor was significant, contrasts based on the *a priori* hypotheses that (a) population densities in the quadrants II + IV differed from those in the quadrants I + III (different soil quality), (b) treatment A was the most efficient treatment (expellant + handsorting) and (c) treatment B was more efficient than C or D (no deleterious effects of CN) were tested.

2.2.2. Frequency distribution

Data from experiment 1 (treatments B, C and D for adults and A, B, C, and D for juveniles) were used to test statistical distributions. For the adults, quadrants I + III and quadrants II + IV were analyzed separately. Measured distributions were compared to a Poisson distribution by χ^2 -test (ELLIOTT, 1977; LINDER & BERCHTOLD, 1979; ZAR, 1984) and, if the test revealed significant differences, to a negative binomial distribution. The parameter k of the negative binomial distribution was determined iteratively by the method of maximum likelihood using equation [3] (LINDER & BERCHTOLD, 1979):

$$\sum_{x=0}^M \frac{F_x}{k+x} = N \ln \left(1 + \frac{\bar{x}}{k} \right) \quad [3]$$

where

x : frequency class,
 M : number of frequency classes,
 F_x : $f_{x+1} + f_{x+2} + \dots + f_M$,
 f_x : observed frequency in class x ,
 N : sum of all f_x ,
 $\bar{x}$: mean numbers of juveniles per 0.25 m²,
 k : parameter of the negative binomial distribution.

2.2.3. Sampling statistics

A stratified random sample with proportional allocation, but where the strata are ignored for the calculation of the variance, resembles a simple random sample with respect to the variance (COCHRAN, 1977). Furthermore, the gain in precision due to stratification (design effect) is assumed not to be important with few strata and only small differences between the strata. Therefore, for the sake of simplicity the optimum sample size for a desired reliability was estimated assuming a random sampling.

The ratio between standard error and the mean is used as an index of reliability (C in equations [4] and [5]). This criterion is commonly used in research work, while in decision making it is more frequent to express the reliability in terms of probabilistic statements (RUESINK, 1980). The optimum sample size for estimating population densities was calculated at given levels of reliability ($C = 0.1$, $C = 0.2$) according to equation [4] for Poisson distributed data and according to equation [5] for negative binomial distributed data, as suggested by KARANDINOS (1976):

$$n = \frac{1}{\bar{x}C^2} \quad [4]$$

where

n : sample size,
 C : proportion defined as the ratio of the standard error to the mean,
 $\bar{x}$: mean numbers of adults per 0.25 m²,

and

$$n = \frac{\frac{1}{\bar{x}} + \frac{1}{k}}{C^2} \quad [5]$$

where

n : sample size,
 C : proportion defined as the ratio of the standard error to the mean,
 $\bar{x}$: mean numbers of juveniles per 0.25 m²,
 k : parameter of the negative binomial distribution.

3. Results and discussion

3.1. Effects of quadrants and treatments

Mean numbers of *L. terrestris* per 0.25 m² and standard deviations were given for each quadrant and treatment in table 2. The population density of the juveniles did not differ between quadrants in the summer (tab. 3), but in autumn 1986 juveniles were more abundant in the quadrants I + III than in the quadrants II + IV. In summer and autumn 1986 the population density of the adults was higher in the quadrants I + III than in the quadrants II + IV (tab. 3). The differences between the quadrants may be due to different soil properties, as described earlier.

For juveniles the choice of treatment had no influence on the efficiency of the extraction in both experiments (tab. 3). Juvenile animals are usually more susceptible to deleterious effects of chemicals than the adults. Considering that juvenile *L. terrestris* were sampled equally efficiently with treatments A (CN/handsorting), B (CN), C (formaldehyde) and D (formaldehyde), it appears that deleterious effects of the expellants do not influence the sampling efficiency at the concentrations chosen. However, the error sum of squares was 87.9% of the total (tab. 3) and the power to prove differences was small.

No difference was found for the adults between the population density estimates obtained by treatments B, and C and D (tab. 3). Hence, CN was not superior to formaldehyde. Treatment A was the most efficient extraction method for the adults. The minimum detectable

Table 2. Numbers per 0.25 m² of *Lumbricus terrestris*, estimated with treatments A to F in the quadrants I + III, and II + IV (means and standard deviations).

treatment	adults		juveniles	
	quadrants I + III	quadrants II + IV	quadrants I + III	quadrants II + IV
A	8.6 (3.53)	6.4 (3.37)	19.0 (6.05)	20.0 (9.29)
B	6.3 (2.50)	5.0 (1.33)	18.6 (8.90)	16.5 (8.19)
C	6.7 (3.09)	4.3 (3.41)	20.2 (7.94)	18.8 (7.98)
D	5.9 (1.91)	3.5 (2.41)	18.9 (6.31)	17.5 (7.85)
E	7.8 (3.49)	8.4 (3.27)	20.7 (7.82)	14.6 (4.65)
F	11.9 (3.78)	8.5 (3.34)	20.0 (5.08)	19.0 (7.59)

Table 3. Relative importance of quadrants and treatments for estimated numbers of adult and juvenile *Lumbricus terrestris*, as revealed by two-factor analyses of variance.

effects of factors	SS of factors as % of the total SS			
	experiment 1 (DF = 79)		experiment 2 (DF = 39)	
	adults	juveniles	adults	juveniles
quadrants	13.8**	5.5 ^{NS}	18.2*	29.3**
I + III vs II + IV	13.3***		3.9 ^{NS}	19.6**
treatments	10.8*	1.8 ^{NS}	8.2 ^{NS}	2.6 ^{NS}
A vs B + C + D	8.6**			
B vs C + D	1.2 ^{NS}			
interactions	4.8 ^{NS}	4.8 ^{NS}	7.7 ^{NS}	3.8 ^{NS}
error	70.6	87.9	65.9	64.2

Explanations: SS = sum of squares; DF = degree of freedom. Symbols for degrees of probability of error: (***) $p \leq 0.001$; (**) $0.001 < p \leq 0.01$; (*) $0.01 < p \leq 0.05$; (NS) $p > 0.05$.

difference (ZAR, 1984) between treatments for the adults in experiment I was calculated to be 11–15% of treatment A.

It is assumed that measures which improve the exposure of the earthworms to the irritant substance enhance the efficiency of the extraction. Earthworms can close the burrows near the surface with their casts. Furthermore, air may be trapped below the penetrating water due to a low air conductivity in moist soil layers near the bottom of the channels. FLÜHLER *et al.* (1986) mentioned discrete entrapped air volumes in the soil of a few microliters to more than 1000 liters and air pressures ranging to 3 kPa above atmospheric pressure after flood irrigation. Such physical constraints can prevent an expellant from getting into contact with the earthworms. Therefore, in a second experiment measures which might improve the exposure of adult *L. terrestris* to the expellant were tested. *L. terrestris* has circadian depth preferences, being near or at the surface at night (JOYNER & HARMON, 1961), therefore samplings were carried out in treatment F after sunset for comparison with daytime sampling. Furthermore, sample units, including an outer border, were ponded intermittently to improve the wetting of the soil. Formally, there was no significant difference between treatment F and E (tab. 3). However, a p-value of 0.054 indicates, that by the very time consuming measures the efficiency of CN as an expellant may have been improved.

The electrical method of THIELEMANN (1986) is also time consuming and too little is known about its efficiency, especially for deep burrowing earthworms. Handsorting is the most efficient earthworm extraction method, but requires by far the greatest amount of time. The choice of an adequate extraction technique remains difficult and is determined by the aims of the study and by variables such as soil type, soil depth and earthworm species (LEE, 1985).

3.2. Frequency distributions

The data on the adults in both quadrants I + III and quadrants II + IV could be described by Poisson distributions (χ^2 , $p > 0.999$).

A negative binomial distribution was chosen to describe the frequency distribution of juveniles, because the observed juvenile distribution differed significantly from a Poisson distribution (χ^2 , $p < 0.001$). The parameter k of the negative binomial distribution was estimated to be 6.1998. A χ^2 -test revealed no significant differences ($p > 0.999$) between observed and theoretical negative-binomially distributed values. Hence, juveniles are probably more contagiously distributed than adults.

To make further inferences on the spatial distribution of *L. terrestris* from frequency distributions is questionable, since the observed variance and distribution may depend on the sample unit size (e.g. WILSON *et al.*, 1989). In earthworm studies it is common practice to transform counts per 1/16, 1/8 or 1/4 m² to population densities per 1 m², and variances are calculated with the transformed data. However, the influence of the sample unit on the proportion of the mean to the variance is generally not known, and therefore, statistical treatment of transformed data may be hampered.

3.3. Sampling statistics

Optimum sample size is population density dependent (fig. 1 and 2) for both adults and juveniles. For a juvenile population density of 20 individuals per 0.25 m², which is about that observed in the investigated meadow, from equation [5] a sample size of 6 is sufficient to reach a desired reliability of $C = 0.2$. The adults were less abundant than the juveniles (tab. 2). Their optimum sample size was 5 from equation [4], assuming a population density of 6 individuals per 0.25 m² and $C = 0.2$. Thus, population density estimations with a reliability $C = 0.2$ may be carried out with little sampling effort. Of course population density fluctuates with time and this has to be taken into account for planning samplings.

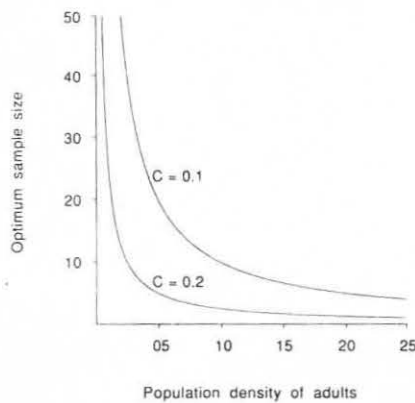


Fig. 1. Dependency of the optimum sample size at given levels of reliability (C) on the mean population density (numbers per 0.25 m^2) of adult *Lumbricus terrestris* in a meadow.

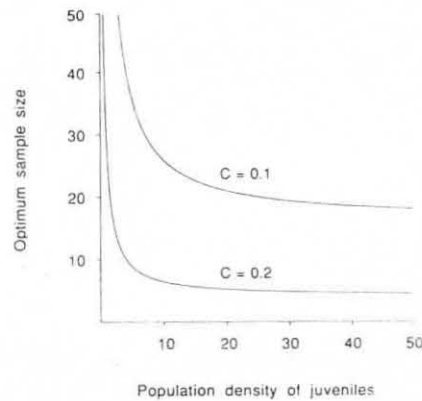


Fig. 2. Dependency of the optimum sample size at given levels of reliability (C) on the mean population density (numbers per 0.25 m^2) of juvenile *Lumbricus terrestris* in a meadow.

For further investigations a sample size of 10 was considered to allow reliable estimates even if the population densities fall to about one half for adults and one quarter for juveniles of those reported here.

Population densities of adult and juvenile earthworms may only be high enough in meadows and some deciduous forests to allow reliable ($C = 0.2$) estimates with low sampling effort. Many other habitats have lower population densities or are less homogeneous than that in this study. In the latter situation, stratified sampling might be required for reliable estimates of the population density. Sample sizes leading to reliabilities of $C = 0.05$ as recommended by SOUTHWOOD (1978) or of $C = 0.1$ as exemplified in fig. 1 and fig. 2 will rarely be taken in field studies on earthworms, since destructive extraction methods such as handsorting, formaldehyde and chloroacetophenone application, or electrical extraction methods are too time consuming for a large sample size. Therefore, attempts to modify sampling methods for earthworms should not only concentrate on how to improve or control the extraction efficiency, but should also aim to reduce the time needed for the sampling.

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